Exhibit 2

B cells move to centre stage: novel opportunities for autoimmune disease treatment

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Abstract | The B-cell arm of the immune system has long been appreciated for its crucial role in pathogen resistance, but in the study of many autoimmune diseases, T cells have dominated the limelight for decades. However, the development of the B-cell-depleting antibody rituximab as a lymphoma therapy has provided a tool to probe the contribution made by B cells in several immune disorders. Recently, the success of B-cell depletion with rituximab in the treatment of rheumatoid arthritis has stimulated investigation of its effects in several other immune disorders, and considerable interest in the potential of drugs that can modulate B-cell function for the treatment of such diseases in general. This article discusses the role of B cells in a range of autoimmune disorders, including rheumatoid arthritis and systemic lupus erythematosus, and analyses approaches to therapeutic B-cell manipulation.

Rheumatoid factors (Rf)
Antibodies capable of recognizing soluble immunoglobulins. These antibodies represent a form of autoreactivity and are prevalent in rheumatoid arthritis and SLE.

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Many human diseases involve exaggerated or inappropriate responses by the immune system. Given the complexity of the tasks that the system performs — exact recognition of a foreign substance, explosive immune-cell replication followed by tissue repair, quiescence and finally the generation of a lasting memory of the event — the potential for an unbalanced response is substantial.

The ability of the immune system to distinguish 'self' from 'non-self' components is crucial, as the aberrant recognition of self components results in damaged tissues and autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus (SLE), Sjögren's syndrome and multiple sclerosis. Attempts to dissect the complex pathogenesis of these diseases in animals have indicated that T cells, B cells, innate recognition systems, pathogenic antibodies, complement and effector cells capable of recognizing the bound antibody can all have major, and often interweaving, roles. Understanding these diseases is further complicated by susceptibility genes, aetiologies that are linked to environmental factors and exposure to infectious agents, and the need to distinguish between immunological mechanisms that contribute to the emergence of disease versus those components that drive established disease.

Successful drugs can also provide key insights into complex human diseases such as rheumatoid arthritis. This disorder was historically considered to have a major B-cell component based on the presence of rheumatoid factors (Rf), but in the 1980s and 1990s attention shifted to the T cell as the crucial element. Recently, however, the ground-breaking work of Edwards and colleagues, who tested rituximab (MabThera/Rituxan; Biogen Idec/Genentech) — a B-cell-depleting antibody developed to treat B-cell-lymphomas — in rheumatoid arthritis[1,2], has triggered a major shift in thinking about this disease. Surprisingly for many, rituximab was found to be effective in the treatment of rheumatoid arthritis[1], and it has now received regulatory approval for this disease. This success, as well as indicating that removal of the B-cell component is beneficial in rheumatoid arthritis, has ushered in a new era of exploration of the contribution of B cells to a range of immune disorders[2–4]. Here, after briefly providing an overview of the relevant aspects of B-cell biology, I discuss studies that are helping to define the contribution of B cells to the pathogenesis of immune disorders such as rheumatoid arthritis, SLE and Sjögren's syndrome, which are stimulating the development of novel drugs with the potential to achieve long-lasting disease remission.

Life as a B cell

B cells have a complex life cycle, and it is important to consider their developmental pathways to understand the ramifications of different intervention points. B cells are routinely divided into two lineages, called B1 and B2 (FIG. 1). B1 cells are long-lived, emerge early in development,

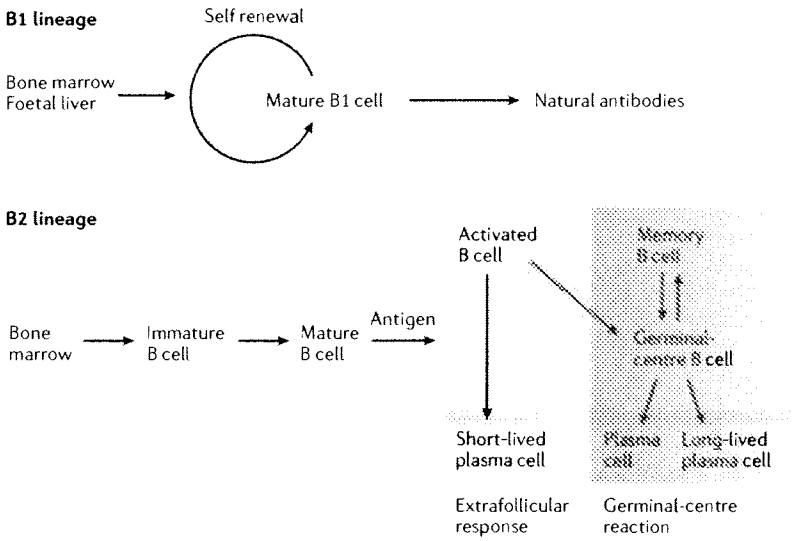

Figure 1 | **B-cell lineages.** B cells are defined in terms of two lineages, called B1 and B2. The figure shows a conventional scheme for the development of these two lineages in the mouse. B1 cells, which are long-lived and self-renewing, produce 'natural antibodies' that are crucial for defence against encapsulated bacteria (human B1 cells are poorly characterized compared with the mouse because of the absence of specific markers; however, B1-cell-derived natural antibodies are present). B2 cells are the more adaptive component of the B-cell system. Immature B2 cells emerge from the bone marrow and undergo maturation primarily in the spleen. Mature B2 cells are activated upon encountering antigen, expand and generate short-lived plasma cells. Some activated cells enter into the germinal-centre reaction that allows for the generation of both memory cells and long-lived plasma cells. Long-lived plasma cells populate the bone marrow.

are self-renewing, and occupy the peritoneal and pleural cavities. They produce polyreactive immunoglobulin M antibodies (IgMs) known as natural antibodies that are considered essential for defence against encapsulated bacteria, and do not undergo extensive somatic hypermutation[5]. There are two types of murine B1 cells that recently have been shown to mediate different functions[6]. In humans, the functions of B1 cells are poorly characterized; however, the output of B1 cells — the natural antibodies — are clearly present. Similar 'innate' B cells have been described whose numbers are increased in systemic lupus erythematosus (SLE)[7].

In contrast to B1 cells, B2 cells have the capacity to generate hypermutated antibodies, and comprise the more adaptive part of the B-cell system. In a program somewhat similar to T-cell development in the thymus, B2 cells emerge and differentiate in the bone marrow, where there is a checkpoint for removal of autoreactive cells (central tolerance)[8]. The immature survivors with functional B-cell receptors leave the marrow and migrate to the spleen, where further selection occurs during the transitional stage (peripheral tolerance). Cells at this point are routed into either a mature follicular B cell or a marginal zone B cell (MZ-B) program. Follicular B cells traffic throughout the secondary lymphoid organs and form the core element of the adaptive humoral response. MZ-B cells are specialized to reside in a compartment that samples the blood stream for pathogens. In many ways, B1 and MZ-B cells are similar, being key parts of the portion of the immune system that is designed to quickly activate and respond to pathogens in the blood as well as the peritoneal and pleural cavities[9,10].

Naive follicular B cells encounter antigens in the secondary lymphoid organs and, in conjunction with cognate T-cell help, become activated and proliferate[11]. Activated B cells can differentiate into plasmablasts that become short-lived plasma cells[12]. This conversion occurs in mice in the spleen at the boundary of the red and white pulp regions at so-called bridging channels. Rodent short-lived plasma cells last 2–3 days and exist to provide an immediate response to a pathogen[11]. The entire initial response leading up to activated B cells, focal expansion and the generation of short-lived plasma cells is termed the extrafollicular response, as it occurs outside of the B-cell follicles[13]. Typically, this phase lasts about 3–7 days. Plasma cells make considerably more immunoglobulin than at the plasmablast stage. Plasma cells are believed to be terminally differentiated and seem to be metabolically adapted for the singular purpose of secreting massive amounts of immunoglobulin[12].

The extrafollicular reaction is followed typically by migration of the activated B cells into the B-cell follicle. There, they coalesce into a tight structure called a germinal centre[14]. The structure is nucleated by a highly specialized reticular network of follicular dendritic cells (FDCs). During the germinal-centre reaction, the cells undergo extremely rapid proliferation, with typically only a couple of B cells giving rise to one germinal centre. The progeny of the germinal-centre reaction differentiate into either plasma cells or memory B cells. Plasma cells derived from germinal-centre reactions shift their display of chemokine receptors leading to egress from the spleen[15]. They then lodge in the bone marrow and are typically long-lived at this point; estimates of their lifespan in mice range from 6–12 months, but could be considerably longer.

How B cells can be bad

The normal beneficial actions of B cells can have a negative side, and FIG. 2 illustrates these aspects[4,16]. Complexation of antigen by antibodies produced by B cells is one of the normal mechanisms for removal of a pathogen or foreign substance. There are many components to the removal mechanism; the recognition of immune complexes by cells bearing Fc receptors and activation of the complement pathway are fundamental. Both of these events lead to the engagement of various effector leukocytes with accompanying inflammation to ensure a robust response to the pathogen; following pathogen clearance, the response is resolved. However, when the substance being attacked is 'self', these same removal mechanisms cannot readily clear the stimulating antigen and chronic inflammation is established, followed by organ damage. Immune complexes can be found in the kidneys in SLE and passive administration of pathogenic antibodies to rodents can induce autoimmune disease in some settings. As circulating autoantibodies can be readily quantified, this area of B-cell function has historically dominated concepts of B-cell involvement in disease.

Natural antibodies
Typically IgM antibodies formed early in development. These antibodies are produced by B1 cells (in mice) and do not display extensive hypermutation. They often have a low affinity for structures on the surfaces of bacteria and often can recognize multiple antigens – that is, they are polyreactive.

Plasma cells
Terminally differentiated B cells essentially dedicated to massive secretion of immunoglobulins. Short (2–3 days) and long-lived (~0.5–1 year) versions exist; long-lived cells reside in the bone marrow and the short-lived cells remain in the splenic red pulp and the medullary cords of the lymph nodes. The gut compartment also has large numbers of plasma cells.

As well as producing appropriate immunoglobulin responses, B cells have other roles, including the presentation of antigen to T cells. B cells can internalize immune complexes, present antigen in the context of class II MHC and display co-stimulatory molecules, and are therefore fully empowered to activate cognate T cells. The interplay between B and T cells is likely to be very important in autoimmune disease. Like their T-cell brethren, activated B cells can express cytokines, and on a per cell basis, B cells can be as efficient at this as T cells[17]. For example, B cells can secrete interferon-γ and interleukin-4 (IL-4) like the T_H1–T_H2 T-cell subsets and they can be skewed in one direction by the corresponding T-cell subset[17–19]. Membrane lymphotoxin-α/β, observed on a subset of B cells, is effectively a cytokine that activates the lymphotoxin-β receptor on some stromal/reticular cells to maintain their differentiation state. These reticular elements contribute to the nucleation and organization of both B-cell follicles within the ectopic infiltrates that can accompany chronic inflammation[20–23]. As the density of B cells in some inflamed sites can be quite high, the contribution of local B-cell-derived cytokine release to an ongoing local autoimmune reaction can be substantial.

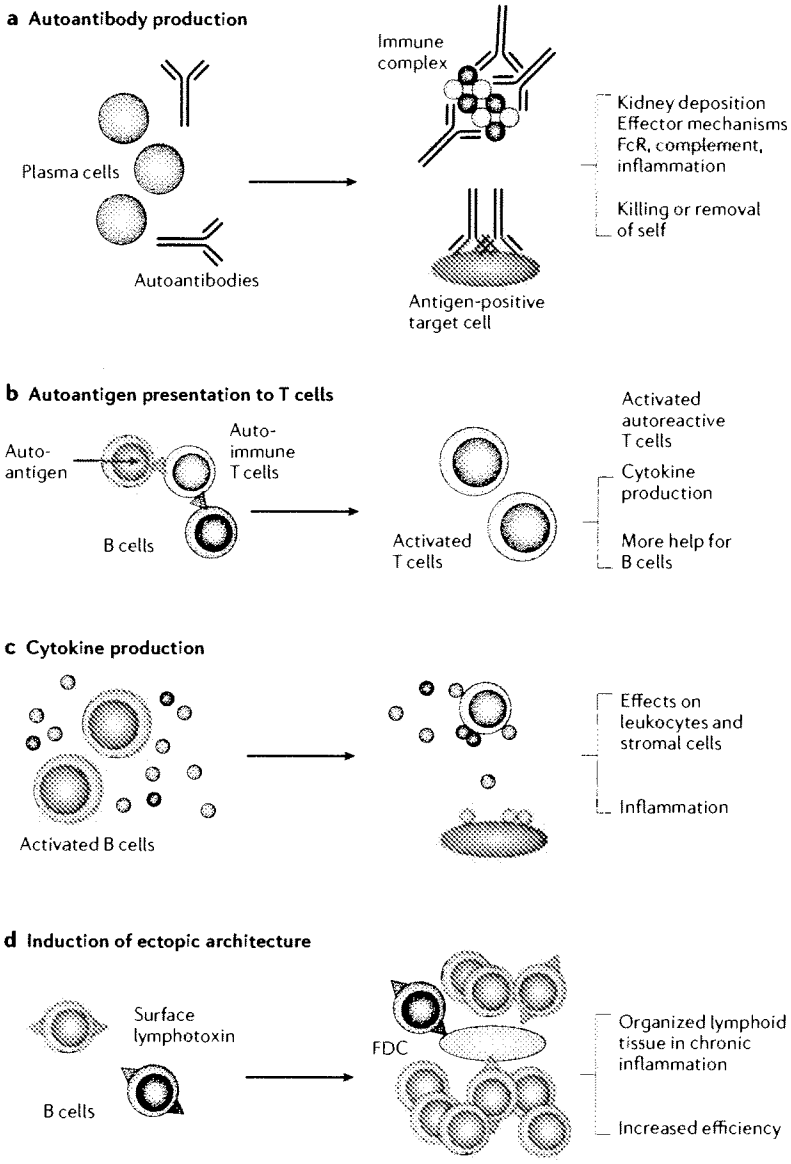

Figure 2 | **How B cells can be bad. a** | B cells produce antibodies that form complexes with target antigens, and the resultant immune complexes can engage B cells, Fc-receptor-bearing effector cells and the complement system. As a consequence, B cells can be co-stimulated and inflammatory processes triggered. In the case of cell-surface immune complexes, effector cells or complement can kill the target cell. **b** | B cells can effectively display antigen and provide co-stimulation signals for T cells that lead to T-cell activation. **c** | B cells release cytokines in a fashion similar to T cells. These cytokines can enhance inflammation and immunological involvement. **d** | The presence of the TNF family member lymphotoxin on the surface of B cells can contribute to the formation of organized lymphoid structures in sites of chronic inflammation. These organized centres most probably enhance immunological involvement. FDC, follicular dendritic cell.

B cells and disease

In recent years, a popular view has been that B-cell derived events are ancillary to a basic breakdown in T-cell tolerance and that although autoantibodies are present, the effector function of T cells is the fundamental pathological component of autoimmune diseases[9]. However, this model is changing even though the aetiology of the various autoimmune diseases in general remains unclear. It is becoming increasingly apparent that the presence of autoreactive antibodies presages the emergence of autoimmune disease in humans[24]. Antinuclear and antiphospholipid antibodies are detected before clinical symptoms in SLE and similar observations have been made for anti-glutamic acid decarboxylase, anti-islet and anti-insulin antibodies in type 1 diabetes[25–28]. Anti-immunoglobulin (Rf) and citrullinated peptide antibodies are seen in early rheumatoid arthritis and antimyelin antibodies can be a harbinger of multiple sclerosis[29–33]. In SLE, maternal transfer of autoantibodies to the foetus can directly induce neonatal lupus syndromes and similar occurrences are observed in myasthenia gravis[34,35]. Likewise, there is some correspondence between adolescents with autoimmune thyroiditis and exposure to maternally transferred autoantibodies, although a genetic predisposition could also underlie the association[36]. Maternal transmission of autoantibodies is also crucial for the development of diabetes in non-obese diabetic (NOD) mice[37].

Although these data are compelling in certain settings, the question of whether autoantibodies contribute directly to early disease or simply correlate with the presence of autoreactive B cells remains largely unresolved[38,39]. Rodent models of arthritis and type 1 diabetes provide support for early B-cell involvement[33,40,41]. In humans, roughly 50% of the B cells emigrating from the bone marrow produce autoreactive and polyreactive antibodies, yet only a few autoreactive cells are present in the mature B-cell compartment[42]. Therefore, the checkpoints are normally very efficient and rather small disruptions in these processes can be sufficient to induce an autoreactive state[6]. In young SLE patients, 25–50% of the naive mature B cells produce autoreactive antibodies, indicating that there is an early defect in tolerance[43]. Rheumatoid arthritis patients exhibit similar defects[28]. On the basis of animal models, it has been proposed that

autoantibodies can be nucleating events for organ-specific pathology[24,40]. These autoantibodies are often not pathological by themselves; however, secondary triggers could push the benign response into self-sustaining chronic inflammation. Infections and innate inflammatory responses might be precipitating events, and in some cases epitopes on infectious agents can directly mimic endogenous self-molecules[44,45]. It is generally accepted that some autoimmune diseases are further driven by autoantibodies once chronic disease is established. Myasthenia gravis, pemphigus vulgaris, Grave's disease and autoimmune thrombocytopaenia are good examples of conditions in which pathogenic antibodies drive the clinical phenotype[4]. SLE is another case in which a constellation of autoantibodies is likely to contribute to a range of systemic and organ-specific events; for example, neuropsychiatric disturbances were associated with the presence of autoantibodies to *N*-methyl-D-aspartate (NMDA) receptors[46]. TABLE 1 lists many of the human diseases with a known B-cell linkage.

Although the direct pathogenic effects of immune-complex deposition have historically been considered to be one of the central elements of autoimmune diseases, more recent work has begun to highlight the role of the antigen-presenting capabilities of B cells and their capacity to secrete cytokines[4]. A precise understanding of the contribution of these elements is crucial for the optimization of B-cell therapies. In SLE, Shlomchik and colleagues have proposed an amplification loop in which autoantibody production and B-cell antigen-presenting capability is entwined with the T-cell arm[47,48]. In a number of elegant studies, engineered mice have provided clear-cut examples in lupus, arthritis, diabetes and fibrosis that B cells are required for disease development, but that their antibody-secreting capability is not[41,49–51]. Moreover, in an experimental model, the antigen-presenting function of B cells was essential for the induction of severe arthritis[50]. In human disease, the contributions made by autoantibodies, antigen presentation and cytokine secretion can vary, a factor that potentially underlies disease heterogeneity. This complexity might be important, as the emerging therapies are directed at one or another component and targeting the inappropriate patient subset will yield an erroneous answer. Likewise, monitoring autoantibody levels might not be productive in those settings in which the other aspects of B cells are the crucial drivers. Indeed, levels of anti-DNA autoantibodies and rheumatoid factor are inconsistently linked to disease status in SLE and rheumatoid arthritis[4,52–54]. It could be speculated that in some cases, autoantibodies such as anti-DNA activate innate systems (discussed below) that directly exacerbate disease, whereas in others, the autoantibodies are crucial for the initial development of the pathology, but then make a lesser contribution once chronic disease is established.

Another development has drawn further attention to B cells in the past few years. The immune system tries to create lymph-node-like structures in chronically inflamed ectopic sites by a process termed lymphoid neogenesis. These ectopic centres can be observed accompanying infection, during heart and liver graft rejection, in conditions such as osteoarthritis and in many autoimmune diseases, including rheumatoid arthritis, Sjögren's syndrome, multiple sclerosis, diabetes and thyroiditis[22,23]. Varying degrees of organization are observed, ranging from small lymph-node-like structures with segregated T- and B-cell zones and germinal centres to more diffuse centres[55]. A specialized vasculature that resembles the high endothelial venules in lymph nodes is usually present and is likely to alter the nature of the inflammatory infiltrate[56–58]. The local generation of B cells from germinal centres and the subsequent production of plasmablasts/plasma cells could dramatically exacerbate disease. Indeed, cells producing autoantibodies are found in the ectopic germinal centres in the salivary glands of some Sjögren's patients[59]. These ectopic structures are probably not a unique disease-specific occurrence, but simply one of the organizational consequences of chronic inflammation, much like the granuloma response to persistent infection.

In the field of transplantation, organ rejection is typically divided into two phases, acute and chronic. Acute responses result from host T cells reacting to the graft; however, about 7% of kidney-transplant recipients develop an acute antibody-mediated rejection[60]. Some cases of acute liver allograft rejection have profound B-cell and plasma-cell infiltrates suggestive of an antibody-mediated response[61,62]. Chronic rejection can be driven by both the T- and B-cell arms[60,63]. The role of antibodies in long-term graft rejection has been relatively ignored, but is currently under scrutiny. Antibodies generated by host B cells that recognize the graft MHC class I or II molecules can bind to the graft endothelial cells, leading to complement fixation and vascular damage. Complement deposition is observed, an indicator of immune complex formation. Rejection of kidney, heart, lung and corneal transplants can have anti-donor antibody contributions.

Diseases long viewed as prototypical T-cell-driven autoimmune conditions, such as multiple sclerosis, are now being re-examined. The example of multiple sclerosis is intriguing, as a fundamental diagnostic tool has been the presence of discrete species of immunoglobulins in the cerebrospinal fluid that are derived from a limited number of B-cell clones residing in the central nervous system[64]. Autoantibodies are implicated in primary progressive multiple sclerosis and ectopic lymphoid centres appear in the meninges, especially in secondary progressive multiple sclerosis[65–67]. B-cell activating factor (BAFF, also known as B lymphocyte stimulator (BLyS)) is also found in these settings[68]. Another example is found in fibrosis: patients with systemic sclerosis with diffuse scleroderma show an immunoglobulin signature in gene-expression analyses similar to that defined in a rodent SLE model[69,70]. The presence of autoantibodies in localized scleroderma is well-documented[71]. As in multiple sclerosis, serum BAFF levels are elevated in systemic sclerosis and skin expression of BAFF is also increased in early diffuse cutaneous systemic sclerosis[72]. Moreover, the loss of the B-cell marker CD19, which is involved in regulating B-cell receptor signalling, reduced disease in the tight-skin systemic scleroderma mouse

Extrafollicular response
For 2–6 days following B-cell activation in the presence of T-cell help, B cells undergo rapid proliferation and differentiation, leading to a burst of plasmablasts. Foci containing these cells remain in the T-cell region of the secondary lymphoid tissues. The resultant plasmablasts reside in the red pulp or medullary cords of the lymph nodes and survive for about 2–3 days.

Germinal-centre reaction
A collection of rapidly dividing B cells assembled on a scaffold of reticular cells. Within this nucleus, affinity maturation and class-switching events are optimized, and memory B cells and plasmablasts are generated.

Fc receptors
A family of generally low-affinity receptors that bind the immunoglobulin Fc domain. Oligomerized immunoglobulin is required for effective binding, and so these receptors serve a pivotal role as one of the primary sensors of immune complex formation. The receptors come in activating and inhibitory versions, and the balance between these two functions determines whether there is a response to immune complexes.

Complement
An enzyme cascade triggered by IgG immune complexes, bound IgM, some mannose-containing substances or certain bacterial surfaces. Activation deposits covalently the protein C3b on the antigen or pathogen, thereby marking it for binding by the various complement receptors. In the case of a cell surface, complement activation triggers the assembly of the membrane attack complex that kills the cell by forming pores in membrane.

Co-stimulatory molecules
A central dogma of immunology states that activation of T- or B-cell receptors alone is insufficient to initiate an immune response. Only when activation is accompanied by a second or third signal does cell activation ensue. The membrane receptors and ligands that provide the second and third signals are collectively referred to as co-stimulatory molecules.

Table 1 | **Diseases with B-cell involvement**

Disease	Target organ(s)
Autoimmune diseases	
Rheumatoid arthritis	Joints
Systemic lupus erythematosus	Systemic
Sjogren's syndrome	Salivary gland
ANCA-associated vasculitis	Vasculature
Antiphospholipid syndrome	Vasculature
Idiopathic thrombocytopaenia	Platelets
Autoimmune haemolytic anaemia	Red blood cells
Guillian-Barré syndrome	Peripheral nervous system
Chronic immune polyneuropathy	Peripheral nervous system
Autoimmune thryoiditis	Thyroid gland
Type I diabetes	Pancreatic islet cells
Addison's disease	Adrenal gland
Membranous glomerulonephropathy	Kidney
Goodpasture's disease	Lung, kidney
Autoimmune gastritis	Stomach
Pernicious anaemia	Stomach
Pemiphigus vulgarus	Skin, mucous membranes
Primary biliary cirrhosis	Liver
Dermatomyositis-polymyositis	Skeletal muscle, skin
Myasthenia gravis	Skeletal muscle
Celiac disease	Small intestine
Inflammatory diseases	
Immunoglobulin A nephropathy	Kidney
Henoch-Schönlein purpura	Vasculature, kidney
Chronic graft rejection	Graft
Atopic dermatitis	Skin
Asthma	Lung
Allergy	Skin, lung, gut
Potential involvement	
Systemic sclerosis	Connective tissue
Multiple sclerosis	Central nervous system
Lyme neuroborreliosis	Central nervous system
Ulcerative colitis	Large intestine
Interstitial lung disease	Lung

ANCA, antineutrophil cytoplasmic antibodies.

model, and scleroderma patients overexpress CD19. In mouse models, B-cell deficiency conferred protection from CCl_4-induced liver fibrosis[49]. B-cell involvement in at least some forms of interstitial lung disease and renal ischaemia reperfusion injury has also been suggested[73–75]. On the basis of these various observations, it is quite possible that B-cell biology contributes to the inflammation–fibrosis progression.

Finally, recent studies have also addressed the question of B-cell and antibody involvement in the initiation and growth of solid tumours. Considerable effort has gone into defining the nature of the antibodies that are secreted by B cells in breast tumours[76]. Moreover, B cells might be a crucial element in promoting the inflammatory milieu that promotes epithelial carcinogenesis, and the elimination of B cells even in established colorectal cancer could be beneficial[77,78]. In general, the full spectrum of B-cell involvement in human disease remains incompletely defined.

B-cell intervention strategies

The complexity of B-cell maturation presents many opportunities for therapeutic interventions, each with advantages and disadvantages. TABLE 2 lists some of the more advanced therapeutic approaches to dampening B-cell involvement, several of which are described in some detail in this section. Signalling in B cells involves some molecules that are fairly specific to B cells, such as Bruton's tyrosine kinase. However, to limit the scope of this article, the focus is primarily on therapeutic approaches involving biologic drugs. Likewise, enhancement of B-cell processes has a major impact on the ability to effectively vaccinate, but this topic is not addressed here.

B-cell depletion. The simplest approach attempts to remove all the B cells, preferably after developing in the bone marrow. Use of an antibody to coat B cells can lead to their removal by immune-system mechanisms (FIG. 3). Rituximab — an antibody against CD20, a surface antigen expressed on B cells — embodies this concept, and its success and relative safety in the treatment of B-cell lymphoma provided a major impetus to assess the depletion of B cells in autoimmune disease. As already noted, rituximab therapy showed considerable efficacy in rheumatoid arthritis[1], leading to FDA approval for rituximab in this condition. Rituximab is being investigated intensively and varying degrees of efficacy have also been reported in a wide range of autoimmune diseases, including idiopathic thrombocytopaenia, IgM-mediated polyneuropathy, Factor VIII deficiency, SLE, Sjögren's syndrome, inflammatory myositis, pemphigus vulgaris, neuromyelitis optica and ANCA-associated vasculitis[2,4,54,79–88]. The next 2–3 years should see significant progress in defining the spectrum of diseases tractable to rituximab therapy.

Following injection of anti-CD20 antibody, antibody-coated B cells in the periphery are rapidly depleted to very low levels, probably as they pass through the liver. B cells within the lymph nodes and spleen are also removed; however, in experiments in monkeys, the depletion of both memory and germinal-centre B cells was less efficient[89]. The actual depleting mechanism seems to be a combination of antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC) and direct induction of apoptosis; and although ADCC is apparently the dominant factor, at least in mouse models, different mechanisms might be engaged within specific compartments[90–93]. Anti-CD20 binding might shift CD20 into a lipid-raft environment, thereby altering calcium flux and inducing apoptotic events[94]. B-cell numbers in the blood remain low for about 6–12 months[95]. Stem cells in the bone marrow are spared, and therefore the generation of naive B cells is retained.

Table 2 | **Strategies for modulating B-cell function in autoimmune disease**

Target	Agent	Effect	Current status
FcR, FcRn	IVIG	Block immune complex signalling	Approved therapy
IgG	Protein A-based absorbants	Remove pathogenic serum IgGs	Approved therapy
IL-6	Anti-IL-6R	Block B-cell differentiation	Approved Castleman's disease Phase III arthritis
CD20	Anti-CD20	Depletion of most types of B cells	Approved NHL, RA Phase II/III autoimmune disease
Anti-DNA antibodies	Injection of DNA-containing scaffolds	Remove anti-DNA pathogenic antibodies	Phase III
BAFF/BLyS	Anti-BLyS	Block BAFF/BlyS survival signals	Phase II RA, SLE
~~BAFF/BLyS~~	~~BR3-Ig~~		~~Phase I RA~~
BAFF/BLyS	AMG 623		Phase I RA, SLE
BAFF/BLyS	Anti-BR3		Preclinical
BAFF/APRIL	TACI-Ig		Phase I RA, SLE
CD22	Anti-CD22	Block CD22 survival signal, cell depletion	Phase III SLE
Lymphotoxin-β receptor (LTβR)	LTβR-Ig	Block ectopic architecture, disrupt germinal centres	Phase II RA
Type I interferon	Anti-interferon-α	Block plasma cell production, other actions?	Phase I SLE
CD40–CD40L	Anti-CD40	Block T–B help	Preclinical
	Anti-CD40L	Block T–B help	Preclinical

BAFF, B-cell-activating factor of the tumour-necrosis factor family; BLyS, B lymphocyte stimulator; BR3, BAFF receptor; Ig, immunoglobulin; IL-6, interleukin-6; NHL, non-Hodgkin's lymphoma; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

Lymphotoxin system
Within the TNF family, the lymphotoxin-α/β ligand binds to the lymphotoxin-β receptor on a subset of specialized stromal or endothelial cells to maintain their differentiation status. Constitutive lymphotoxin-β receptor signalling is required to maintain various micro-environments such as the polarized B-cell follicles, follicular dendritic cells and high endothelial venules.

Non-obese diabetic mice
(NOD mouse). A strain of autoimmune-prone mice that spontaneously develops diabetes due to an autoimmune attack on the pancreatic islet cells. This mouse serves as a common model for autoimmune disease and specifically human type I diabetes.

Lymphoid neogenesis
Within chronically inflamed tissues, leukocytic infiltrates can organize into an ectopic structure that resembles a lymph node. Aggregates of T and B cells, macrophages and dendritic cells assemble and specialized 'high endothelial venules' develop, allowing for additional trafficking opportunities. In the most organized cases, T cells segregate spatially from B cells, and germinal-centre reactions form within the B-cell follicle.

Antibody-dependent cellular cytotoxicity
(ADCC). Antibody-coated cells can be recognized by Fc receptors on natural killer cells and macrophages. Receptor engagement leads to direct killing of the coated cell by release of various agents.

Complement-dependent cytotoxicity
(CDC). Complement components assemble on complexes between antibodies and antigen (immune complexes). The assembly culminates with formation of the membrane attack complex, which directly creates pores in the membrane surface and kills the cell.

Because plasma cells are not depleted, serum immunoglobulin levels derived from long-lived plasma cells in the bone marrow are maintained, which might be a crucial feature of this therapy.

Which types of B cells are removed by rituximab therapy and their relevance to clinical efficacy is a question receiving considerable attention. The answers will reveal much about the nature of the disease process and routes to improving B-cell-based therapies. One area of interest with rituximab concerns the potentially incomplete depletion of B-cell subsets in the lymph nodes and spleen as compared with the blood[93,96]. In mice and monkeys, the recirculating follicular B cells are depleted efficiently, although elimination of germinal-centre B, B1 and MZ-B cells is incomplete. This possibly results from non-optimal micro-environments that are capable of mediating ADCC or CDC, or because these cells do not readily undergo apoptosis in response to treatment[4,89,93,96,97]. The impact of rituximab therapy on B-cell memory is currently unclear. The sensitivity of the various B-cell subsets to rituximab, and inhibition of BAFF and the CD40 pathway, is summarized in TABLE 3. In rheumatoid arthritis, disease can relapse 6–12 months post-treatment at a point when B-cell levels are being re-established[98]. Either the entire autoimmune repertoire is not depleted and the system restores to the original state, or new naive bone-marrow-derived autoreactive B cells are not efficiently removed at the tolerance checkpoints and there is an efficient reconstitution of the autoimmune state. A better understanding of this process could lead to more sophisticated strategies, perhaps resulting in more robust remission.

Examination of autoantibody titres also sheds some light on these events. In rheumatoid arthritis, Rf titres decrease about two- to threefold following depletion, which is suggestive of a short-lived plasma cell source for much of this Rf[99,100]. Therefore the memory cells generating these short-lived plasma cells must be affected, leading to the reduction in serum titres. Whether this decrease is sufficient to reduce symptoms is an open question, although some correlation with serum levels of C-reactive protein and disease status was noted[99]. In SLE, more substantial reductions were observed in autoimmune anti-DNA titres in a subset of patients, and the elevated plasma cell numbers in the blood normalized[95]. These data are also consistent with impaired generation of short-lived plasma cells. When examined, the reconstituting B cells seemed to be naive and to have a new and diverse immunoglobulin rearrangement pattern, although contributions from the resistant original mature compartment were found[98,101]. Work in rodents has implicated both the MZ-B and B1 compartments in certain autoimmune settings and the MZ-B compartment might be relatively large in humans[9,102]. The contributions of these two compartments to human disease are relatively unexplored[10].

Another potentially important factor is the contribution of long-lived plasma cells in some disease settings. The replenishment of short-lived plasma cells can be blocked by depletion of the mature B cells. However, long-lived plasma cells are resistant even to radiation and metabolic poisons (for example, cyclophosphamide), and this compartment currently lacks an effective intervention strategy[103,104]. If there are patients in

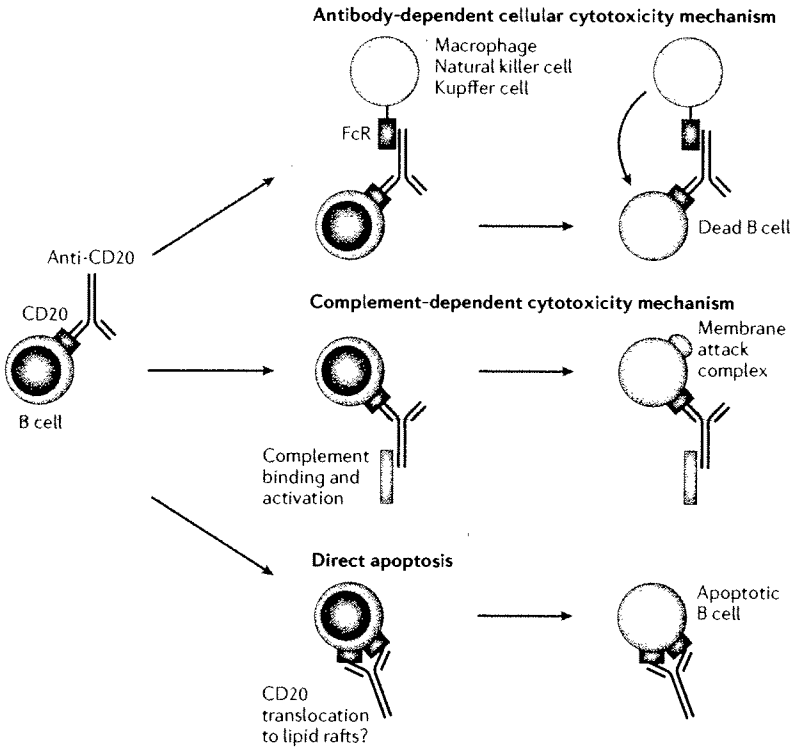

Figure 3 | **Mechanism of action of anti-CD20 antibodies.** B cells coated with anti-CD20 are killed by a cell-based mechanism called antibody-dependent cellular cytotoxicity (ADCC). Alternatively, the complement membrane attack complex is assembled on the cell surface in a process called complement-dependent cytotoxicity (CDC). Antibody binding can force CD20 into lipid-raft environments, resulting in altered calcium flux with apoptotic consequences.

whom these cells are major disease drivers, the efficacy of most B-cell therapies will not manifest itself within a reasonable window for clinical experimentation (that is, within a year). In mice that spontaneously develop an SLE-like disease, long-lived plasma cells were found to exist in the spleen[103]. This is an unusual location for these cells that is suggestive of the aberrant establishment of survival niches for these cells in a pathological state[103]. The retention of the original oligoclonal immunoglobulin banding patterns in the cerebrospinal fluid of multiple sclerosis patients following stem-cell transplantation suggests population of the central nervous system compartment by long-lived plasma cells[105-107]. An understanding of whether certain autoimmune diseases, or disease in some patient subsets, are driven by systemic short-lived plasma cell production or niches of long-lived plasma cells could contribute to the design of more effective therapies. The ability to purge the system of both B cells and long-lived plasma cells, followed by repopulation with a naive repertoire, could be very useful and less radical than bone-marrow transplantation, although this approach is potentially fraught with weakened host resistance.

It is likely that the current rituximab therapy can be improved[92]. One variable lies in the polymorphisms found in the Fc receptors on the cells that are crucial for ADCC-mediated depletion. Studies in follicular lymphoma, Waldenstrom's macroglobulinaemia and SLE revealed that polymorphisms that rendered that receptor less efficient in binding to rituximab led to decreased efficacy in those patients[108-111]. Such factors and others perhaps unique to SLE patients, such as lower levels of complement components, might contribute to variable depletion efficiencies. More efficient harnessing of human Fc receptor engagement might be beneficial. Other abundant B-cell antigens might be candidates to eliminate B cells; for example, the anti-CD22 antibody epratuzumab is being explored for its effects on B-cell survival, and an anti-CD19 antibody has been shown to deplete B cells effectively in mouse models[112,113]. An antibody to CD52 (alemtuzumab; CamPath) represents a more complex case that depletes subsets of both T and B cells.

Manipulation of B-cell survival. Discovery of B-cell activating factor (BAFF, also called B lymphocyte stimulator (BLyS)), opened a new avenue to the manipulation of B cells and this topic has been extensively reviewed[114-117]. In mice, all B cells from the late transitional stage through to the germinal-centre B cell require BAFF-induced signalling through the BAFF receptor for survival. BAFF levels are elevated in the blood of patients with diseases such as rheumatoid arthritis, SLE and Sjögren's disease, and it was therefore logical to conclude that excess BAFF might contribute to the expansion of certain B-cell compartments, although the mere presence of excess BAFF is unlikely to initiate autoimmune disease. There is evidence that BAFF might also participate in B-cell differentiation and aspects of T-cell function in addition to its basic survival function[118]. Several approaches are being taken to inhibiting BAFF function (FIG. 4). An anti-BAFF antibody called belimumab has been studied in SLE and rheumatoid arthritis, with some efficacy in rheumatoid arthritis reported by Human Genome Sciences. BAFF has three receptors, BAFF-R (also called BR3), TACI and BCMA. The BAFF receptor–Ig fusion protein (BR3–Ig) inhibits only BAFF and not a closely related ligand called APRIL. The decoy receptor TACI–Ig is also being explored clinically, and has the potential advantage of blocking both BAFF and APRIL. A fourth potential modality would use anti-BAFF-R antibodies that block BAFF binding. The combined application of these BAFF/APRIL blockers with B-cell-depletion schemes might be attractive.

Sufficient data from monkey experiments now exist to allow a comparison of BAFF inhibition (note this is potentially distinct from simultaneous BAFF and APRIL inhibition) with rituximab. BAFF inhibition decreases the number of B cells by about 50% in both the blood and in the secondary lymphoid tissues; however, the change occurs slowly relative to rituximab[119,120]. The MZ-B cell is more sensitive to BAFF inhibition than rituximab; moreover, neither therapy dramatically reduces memory B-cell or germinal-centre B-cell numbers[89,119]. Like rituximab, BAFF blockers are unlikely to directly affect the survival of plasma cells[121]. The less dramatic reduction in B cells numbers (with relatively slow kinetics) in primates was surprising when compared with mice.

Table 3 | **Comparison of B-cell sensitivity at different intervention points**

B-cell stage	Inhibition of:			
	Anti-CD20	BAFF	BAFF+APRIL	CD40L
B1	?	–	–	?
Pro/Pre	–	–	–	–
Immature	√	–*	–*	–
Follicular	√	√	√	√‡
Marginal zone	√	√	√	–
Germinal centre	–?‡‡	–?‡‡	?	√‡
Memory	?	?	?	√‡
Plasma cell	–§	–§	?¶	–§

*BAFF is required at the late transitional (T2) step during maturation. ‡Anti-CD40L or antagonistic anti-CD40 will block both T-dependent primary and secondary responses. §Interference with memory or extrafollicular B cell responses will reduce the numbers of short-lived plasma cells. ¶Plasma cells might require APRIL–BCMA signalling for survival. ‡‡The effect on germinal-centre reactions remains unclear in primates. APRIL, a proliferation-inducing ligand; BAFF, B-cell activating factor; BCMA, B-cell maturation factor; CD40L, CD40 ligand.

Although laboratory mice possess mostly re-circulating naive follicular B cells, and therefore differ from the memory-rich populations found in mature primates, the pharmacological differences might be suggestive of species differences in the extent of BAFF/APRIL-dependency. Along this line, the field still lacks precise knowledge of the relative roles of APRIL versus BAFF and BAFF-R versus TACI or BCMA in humans. The role of APRIL in istotype class switch has been highlighted, but its function remains relatively ill-defined[114,122]. People with genetically impaired TACI have common variable immunodeficiency, which validates the importance of this receptor to human disease[123,124]. On the basis of mouse studies, TACI was defined as a negative regulatory element, and therefore the human findings clearly call this assertion into question and again highlight potential differences between primates and mice in the factors and receptors controlling B-cell survival[125]. BCMA is expressed by plasma cells, although the question of whether BAFF/APRIL controls the survival of this cell type in humans requires more attention[126]. Given these uncertainties, the consequences of BAFF versus BAFF/APRIL inhibition and the impact of any cell-type selectivity of rituximab treatment versus survival inhibition will have to be resolved in the clinic[93].

T-cell help. Less radical interventions can be envisioned that would target individual steps in the B-cell life cycle and therefore build in more selectivity. Conventional dogma states that because high-affinity autoantibodies in humans show extensive hypermutation, the B cells that produce them must have gone through the germinal centre reaction. Therefore, the germinal centre reaction is a good target for a more selective B-cell therapy. Whether a germinal-centre-specific therapy would be efficacious has not been well-investigated, even in mice. More recent work has shown that hypermutation can occur outside the germinal-centre reaction in the extrafollicular compartment; moreover, in mice some immature B cells emerging from the bone marrow already show signs of hypermutation[127,128]. These observations beg the question of how absolute the linkage between hypermutated clones recognizing autoantigens and the germinal-centre reaction in autoimmune disease is. The autoimmune memory repertoire is effectively established at the time of diagnosis, although in T cells the spectrum of autoreactive epitopes is believed to spread. In established disease, it remains unclear whether the germinal-centre reaction is required to further extend the autoimmune repertoire and to provide for a continual supply of plasma cells, as opposed to memory cells generating plasma cells in non- germinal-centre micro-environments. Nonetheless, germinal centres dominate the B-cell follicles in primates, which provides an efficient environment for class switch and affinity maturation, and makes the germinal-centre reaction a logical intervention point.

One of the more enticing strategies for affecting B-cell function is blockade of CD40 signalling. This pathway is essential for T-cell help to B cells, and hence blocking it prevents B-cell activation and class switching at both the extrafollicular and germinal centre levels. In addition, aspects of dendritic cell function that are CD40-dependent are inhibited. Patients lacking a functional CD40 pathway produce only IgM and not IgG. Furthermore, CD40 blockade can induce transplantation tolerance and offers the potential for long-term engraftment without rejection[129]. In early trials, anti-CD40 ligand (CD40L) therapy showed efficacy in idiopathic thrombocytopaenia and resulted in the improvement of several disease markers in SLE[130–133]. This approach was halted by vascular complications stemming from antibody binding to CD40L displayed on activated platelets. Despite this complication, there is a very compelling rationale to pursue targeting this pathway. The potential removal of the platelet issues through Fc modifications, blocking CD40 receptors with monoclonal antibodies or interfering with specific elements of the signal transduction pathway remain viable modalities.

Other opportunities for the manipulation of germinal centres include CD19 blockade or removal of the FDC networks. CD19/CD21 are important molecules in B-cell activation, and it was recently shown that genetic loss of CD19 signalling disrupts germinal-centre formation[134]. A rather different approach involves modification of immune architecture. The lymphotoxin system is crucial for the maintenance of a number of stromal cells that are integral to the optimal performance of the secondary lymphoid organs. Notably, FDC networks require the lymphotoxin pathway and their disruption will eliminate or abort germinal centre reactions. A decoy receptor, lymphotoxin-β receptor (LTβR)–Ig, which will block surface lymphotoxin forms, is currently in clinical trials[20].

Although outside the scope of this review, manipulation of T-cell function has consequences for B-cell responses. Notably, blockade of B7 binding to CD28 and cytotoxic T-lymphocyte-associated protein 4 (CTLA4) by CTLA4–Ig reduced disease and anti-DNA titres in rodent models of SLE[135]. Abatacept (Orencia; Bristol-Myers Squibb), a human CTLA4–Ig molecule, has been approved for the treatment of rheumatoid arthritis. In addition to CD40 and CTLA4, several members of the tumour-necrosis factor family and other members of the

Affinity maturation
Somatic hypermutation results in altered antibodies and occurs efficiently in the germinal centre. Antibodies with higher affinity for the antigen are selected for as the response progresses.

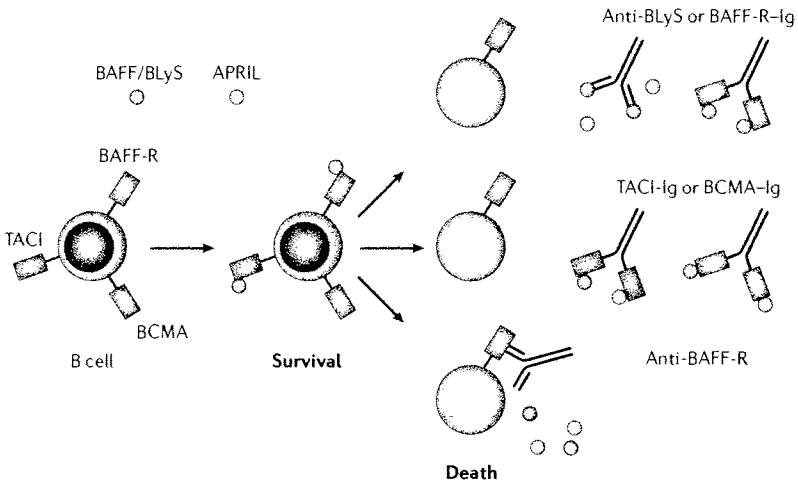

Figure 4 | **Manipulation of B-cell survival.** B-cell activating factor (BAFF/BLyS) can provide a survival signal to B cells via binding to the BAFF receptor (BAFF-R), and both BAFF and a proliferation-inducing ligand (APRIL) can similarly signal through transmembrane activator and CAML-interactor (TACI) and B-cell maturation factor (BCMA). On the basis of rodent studies, BAFF binding to the BAFF-R is believed to deliver the primary survival signal to the B cell. The contribution of the additional ligand APRIL and the other two receptors TACI and BCMA to cell survival is less clear, especially in man. Anti-BLyS and the soluble BAFF-R-Ig fusion protein block BAFF selectively without affecting APRIL. Likewise, anti-BAFF-R blocking antibodies would be selective for BAFF. Soluble forms of the TACI and BCMA receptors will inhibit both ligands and hence potentially a larger swath of biology.

B7 co-stimulation family, such as ICOS, have co-stimulatory roles that might be exploitable in this capacity to modulate B-cell responses[136,137]. Indeed, humans with a genetic defect in inducible T-cell co-stimulator (ICOS) present with immunodeficiency and impaired germinal centre formation[138].

Cytokines. Several cytokines are crucial for B-cell activation and expansion. IL-6 has long been known to drive terminal differentiation of B cells into plasma cells as well as being able to influence a host of other activities potentially relevant to autoimmune disease[139]. Moreover, IL-6 was recently defined as a cytokine found in chronically inflamed environments that in combination with transforming growth factor-β1 (TGFβ1) can induce differentiation of the pathogenic IL-17-producing T-cell subset[140]. In light of these findings, IL-6 might be a pivotal cytokine. Blockade of IL-6 activity with the anti-IL-6 receptor monoclonal antibody tocilizumab has shown efficacy in rheumatoid arthritis, systemic onset juvenile idiopathic arthritis and in Crohn's disease[141–144]. The antibody was recently approved for use in the treatment of Castlemen's disease, a rare lymphoproliferative disease with expansion of plasma cell numbers. Efficacy in a Phase III rheumatoid arthritis study has been noted and therefore targeting IL-6 seems to have considerable potential. IL-21 is also a cytokine with B-cell differentiating activity and as such is a promising target[145].

Another area that has received considerable attention in the field of SLE concerns the role of type I interferon (IFN). SLE patients have elevated levels of circulating IFN. Gene-expression analyses of blood cells and affected target organs from SLE, Sjögren's syndrome, dermatomyositis, psoriatic arthritis and a subset of rheumatoid arthritis patients revealed increased expression of a collection of genes known to be induced by type I IFN — that is, an 'IFN signature'[146]. Plasmacytoid dendritic cells are suspected as the source of the excess IFN. IFN is a pleiotropic cytokine implicated at many levels, two of which stand out. First, IFN can trigger maturation of myeloid dendritic cells that can further enhance adaptive immune responses[147]. Second, IFN in combination with CD40 signalling can cause B cells to differentiate into plasmablasts. In the presence of IL-6, these cells progress further to plasma cells. For these reasons, an IFN-blocking therapy might be useful in SLE and this approach is being pursued clinically.

Toll-like receptors. In parallel with the explosion of interest surrounding the role of IFN in SLE, exciting developments are occurring in the understanding of how autoreactive B cells can be activated. The Toll-like receptors (TLRs) are sensors that respond to components of pathogens and provide the innate signalling that activates dendritic cells and macrophages, as well as playing key roles in B cells. Infectious events can be associated with the onset or exacerbation of autoimmune disease, and TLRs are probably central to this connection. Historically, the role of TLRs in antigen-presenting cell function has been the focus; however, it was a surprise to find that direct TLR activation on B cells is required for efficient B-cell responses (both T-cell-dependent and T-cell-independent)[148]. In addition to the activation of TLR by microbial products, endogenous DNA and RNA forms are able to trigger TLRs[149–151]. In SLE, interest has focused on modified self-antigens that are the products of apoptotic or necrotic events[152]. As one example, TLR9 signalling is required for anti-DNA autoantibody production in a spontaneous murine model of SLE[153]. Plasmacytoid dendritic cells secrete type I IFN in response to anti-DNA–DNA complexes and potentially other endogenous ligands[152,154,155]. Marshak-Rothstein and colleagues have demonstrated that chromatin can be directly internalized by B cells bearing DNA-specific B-cell receptors, allowing presentation to intracellular TLR7/9[152]. Likewise, complexes of chromatin components and anti-chromatin antibodies can be internalized into B cells by B-cell receptors with rheumatoid-factor-type specificity. So, TLR activation by infectious agents or by endogenous ligands could be driving B-cell-centric pathology at several levels, and therapeutic manipulation of TLR signalling is an active area of research.

Effector functions. The effects of immune complexes are mediated in large measure through interactions with Fcγ receptors (FcγR). FcγR are displayed by monocytes, granulocytes, natural killer cells and B cells, and are generally of low affinity such that only oligomeric antibody–antigen complexes bind well. Receptor activation by immune complexes can trigger pro-inflammatory events and modulate the immune system. These receptors come in activating and inhibitory forms, and therefore represent a rich area for therapeutic manipulation[156,157]. The activation

status of many FcγR-bearing cells reflects a balance in signalling from activating and inhibitory receptors. This is nicely illustrated by the SLE-like condition of mice with a deleted FcγRIIB inhibitory receptor; furthermore, excess inhibitory receptor was shown to re-establish tolerance in a rodent SLE model[156,158,159]. This observation was recently paralleled in several polymorphisms associated with SLE in humans. One polymorphism in the human FcγRIIB inhibitory receptor leads to the exclusion of the receptor from lipid rafts and therefore decreased inhibitory capability[160,161]. Likewise, decreased expression is linked to an FcγRIIB promoter polymorphism[162]. In both cases, decreased levels of FcγRIIB inhibition shift the balance towards more activation by immune complexes. High-affinity-binding alleles of the activating FcγRs also increase the risk of developing SLE[53]. Blockade of the activating receptors could reduce the effects of immune complexes; alternatively, schemes to enhance the inhibitory activity of FcγRIIB could dampen the system. This area is exciting and in rapid transition, as the recent delineation of the FcγRIV has brought additional clarity, especially in terms of the capability of various Ig isotypes to engage FcγR[156].

The complement system represents the other major effector pathway and is clearly interwoven with autoimmune pathology[163]. There are three different initiating mechanisms for complement activation, which lead to the engagement of many components and provide a rich field for intervention. However, beyond its role in pathogen defence, complement can have beneficial functions as evidenced by the predisposition towards SLE of people with complement deficiencies. Whether this system, with its positive and negative components, can be modulated chronically in a safe manner remains to be seen.

A common treatment for immunoglobulin-mediated autoimmune disease is to administer large amounts of IgG derived from pooled human donors. There have been many reported explanations for why this agent works, and probably a constellation of events are involved, with effects on FcγR being dominant[156,164]. These observations reinforce the logic for FcγR intervention. Another approach is the manipulation of the immunoglobulin recycling Fc receptor FcRN[165]. This receptor salvages IgG that has been internalized and returns it to the blood. Without this mechanism, the lifespan of IgG in the blood is much shorter, and therefore this component could be manipulated to accelerate elimination of IgG in the blood, including pathogenic IgGs, or conversely to maintain immunoglobulin-based drugs in circulation for longer. Direct removal of serum immunoglobulin by passing the blood over a Protein A-containing matrix (Prosorba column) is used in recalcitrant cases of rheumatoid arthritis[166]. Selective clearance of DNA-reactive antibodies has been explored in SLE using a scaffold with DNA attached called LJP 394 or Riquent[167]. The success of these approaches relies on the premise that circulating pathogenic autoantibodies are dominant drivers of disease in some settings.

Allergic reactions occur following recognition by IgE of various foreign antigens such as food components, drugs, insect bites and inhaled particulates, including dust-mite faeces, pollen and dander. Crosslinking of IgE leads to Fc receptor signalling on mast cells, basophils and eosinophils. Consequently, these cells release mediators that cause the inflammation and smooth muscle and vasculature changes that are characteristic of allergic responses. Many approaches have been developed to blunt the downstream events following IgE responses. Recently, the non-anaphylactic anti-IgE antibody omalizumab (Xolair; Genentech/Novartis) has been successfully used in these settings, representing a new approach to the treatment of allergic disease[168,169]. Manipulation of Fc receptors with negative signalling capability is also being attempted[170].

Safety of B-cell-directed therapies

Diseases such as rheumatoid arthritis and SLE are lifelong and therefore the long-term safety of any chronic therapy is an important concern. Data addressing the safety of B-cell-directed therapies are only available for rituximab exposure, and even here one needs to draw primarily from the substantial experience with haematological malignancies. Rituximab is well-tolerated and generally safe[171]. A key component of the safety of rituximab probably lies in the retention of serum immunoglobulin levels. Serum immunoglobulin is produced by long-lived plasma cells in the bone marrow and 50% of 'natural' IgM is derived from B1 cells, at least in mice[172]. The lack of an impact on serum immunoglobulin titres following rituximab therapy seems to reflect the resistance of both of these compartments to depletion. Whether many rounds of B-cell depletion will eventually erode these populations is under investigation. Other potential safety issues concern the ability to deliver a primary or secondary vaccination to a patient with deficient B-cell function. Other agents that target the germinal-centre reaction or the splenic MZ-B compartment might have their own unique safety concerns.

Concluding remarks

There is an emerging appreciation for the pivotal role played by B cells in several areas of human disease. This concept is now validated by clinical results that have established B-cell-directed therapy as a promising therapeutic strategy for several diseases, including autoimmune diseases such as SLE and Sjögren's syndrome that are poorly served by modern medicine. The B-cell field has found a simple and apparently safe agent in rituximab to survey the range of B-cell involvement in human pathology. Rituximab also provides a comparison point for the assessment of the value of alternative B-cell-directed approaches. A frame of reference is very important to provide some level of reassurance considering the vast resources needed to explore agents at the clinical level. Another fortuitous trend is that the recent awareness of B cells in human disease coincides with the vastly accelerated pace of research on the innate immune system, as well as plasma cells and the factors supporting their survival in various niches. Likewise, the study of B-cell survival factors such as BAFF/BLyS has advanced rapidly, culminating now in clinical proof-of-concept testing.

Challenges to the field include resolving questions about which subsets of B cells fundamentally contribute to established disease, and where they reside. What are the relative roles played by autoantibodies, B-cell presentation and short- versus long-lived plasma cells in established disease? Is truncation of a pathogenic B-cell response early in the course of disease able to induce a more durable remission? Advances have been limited by the heterogenous nature of diseases such as SLE coupled with difficult clinical trials and rather cumbersome animal models[173]. Moreover, when compared with rheumatoid arthritis or multiple sclerosis, the pharmaceutical industry lacks well-vetted metrics for successful trials in SLE and Sjögren's disease. Intense efforts are ongoing for improved biomarkers that could serve as surrogates for efficacy and tools for patient stratification for different therapies. Hopefully, these advances will accelerate clinical exploration and this confluence will result in a wider range of treatment options for these diseases.

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Competing interests statement

The author declares competing financial interests: see Web version for details.

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